Leukotriene B_4 stimulation of phosphatidylinositol turnover in macrophages and inhibition by pertussis toxin

Andrij Holian

Pulmonary Division of the Department of Internal Medicine and Department of Pharmacology, University of Texas Medical School, Houston, TX 77030, USA

Received 1 April 1986

The influence of leukotriene B_4 (LTB_4) on phosphatidylinositol (PI) cycle activity was investigated in the guinea pig alveolar macrophage. In contrast to the observation reported in leukocytes [(1984) Proc. Natl. Acad. Sci. USA 81, 5966–5969], LTB_4 was found to stimulate PI cycle activity in the macrophage (transient decrease in phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 4-phosphate, transient elevation of 1,2-diacylglycerol and elevation of phosphatidic acid) similar to the stimulation of the PI cycle by the chemotactic formyl peptides. Preincubation of macrophages with 1 μg/ml of pertussis toxin, for 1 h at 37°C, blocked LTB_4 and formyl peptide stimulated O_2^- production and PI cycle activity. Therefore, both stimulants are proposed to act through the same coupling protein to activate phospholipase C in the early stages of macrophage activation.

Macrophage *Formyl peptide* *Leukotriene B_4* *Phosphatidylinositol* *Pertussis toxin*

1. INTRODUCTION

Leukotriene B_4 (LTB_4) is a potent inflammatory agent that is synthesized by the lipoxygenase pathway and detected by receptors on phagocytic cells (reviews [2,3]). LTB_4 elicits a variety of responses from leukocytes [4] including production of superoxide anion (O_2^-). However, the biochemical mode of action of LTB_4 has not been completely elucidated. LTB_4 receptors on leukocytes have been demonstrated and two classes of binding sites have been identified [5]. LTB_4 has been shown to cause Ca^{2+} mobilization in leukocytes which has been proposed to be due either to an undefined ionophoric property of LTB_4 [6] or to some other receptor-linked mechanism [1]. LTB_4 stimulation has been shown to be blocked by pretreatment with pertussis toxin [7]. Pertussis toxin is proposed to ADP-ribosylate a 41 kDa coupling protein between surface receptors such as the formyl peptide receptors and phospholipase C [8]. However, LTB_4 stimulation was recently shown not to involve activation of phospholipase C in leukocytes [1].

The reported lack of stimulation of phospholipase C by LTB_4 results in an apparent discrepancy in the model of cellular stimulation through a pertussis toxin inhibitable coupling protein. If LTB_4 stimulation is transduced through a similar pertussis toxin inhibitable coupling protein as is utilized for formyl peptide stimulation and both stimulants cause Ca^{2+} mobilization, then the inability of LTB_4 to activate phospholipase C cannot be readily explained. To clarify this apparent inconsistency in the biochemical pathway of LTB_4 cellular activation we investigated the action of LTB_4 on the phosphatidylinositol (PI) cycle and compared these results with formyl peptide activation. Here, we report that LTB_4 stimulates PI turnover in the macrophage in a similar manner to that observed for formyl peptides. In addition, PI turnover and O_2^- production stimulated by either formyl peptides or LTB_4 can be blocked by pertussis toxin treatment of macrophages.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2. MATERIALS AND METHODS

Alveolar macrophages were isolated from guinea pigs as in [9]. Cells were suspended in Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (MHBSS). Macrophage purity (ranged from 85 to 95%, PMN were always <5%), viability to trypan blue (>93%) and total cell counts were assayed as described [10].

Extracellular levels of superoxide dismutase inhibitable superoxide anion were monitored continuously at 21–23°C as described in [11] using a Perkin-Elmer Lambda 3 recording spectrophotometer by measuring the rate of ferricytochrome *c* reduction (75 μM, type III; Sigma, St. Louis, MO) at 550 nm. An extinction coefficient of 18.5 mM (reduced-oxidized) was used for ferricytochrome *c*.

The flux of $^{32}P_i$-labelled metabolites through the PI cycle was measured as in [10]. Analysis of phosphatidic acid (PA), phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2) was carried out by a modification of the method in [12] as described [13].

The formation of diacylglycerol was measured in macrophages prelabelled with [^{3}H]arachidonic acid. Macrophages (4.5×10^6) were incubated with 10 μCi [^{3}H]arachidonic acid in a volume of 0.5 ml for 1 h at 21–23°C. The cells were then centrifuged and washed once in fresh medium and resuspended to final volume of 3.1 ml in MHBSS. Unstimulated samples (0.25 ml) were quenched with 1 ml of chloroform/methanol/conc. HCl (20:40:1, v/v). LTB_4 (15 nM) was then added and 0.25-ml samples were quenched as described above. The layers were separated by the addition of 0.25 ml each of chloroform and H_2O followed by centrifugation. The upper aqueous layer was vacuumed off and 0.4 ml of the organic layer was transferred to 1.5-ml Eppendorf tubes and the samples dried under reduced pressure using a Speedvac concentrator (Savant Instruments, Hicksville, NY). Each of the dried samples was resuspended in two separate 20-μl aliquots of quenching solvent and each aliquot along with standards (monoolein, 1,2-diolein, 1,3-diolein and triolein (Sigma)) were spotted on the preadsorbent area of HPTLC plates (Linear-K, Whatman, Clifton, NJ) which were prewashed in developing solvent (petroleum ether (b.p. 60–70°C)/diethyl ether/acetic acid (70:30:1, v/v)). The plates were then developed in the same solvent. The standards were detected with iodine vapor and radioactive areas were detected by 24 h exposure to X-ray film (Kodak XAR-5) following spraying with EN^{3}HANCE (New England Nuclear, Boston, MA). The radioactive areas corresponding to 1,2-diolein (R_f = 0.23) were scraped into scintillation vials for counting as described [10].

FNLLP was obtained from Sigma and pertussis toxin from List Biological Laboratories (Campbell, CA). $^{32}P_i$ was purchased from New England Nuclear. LTB_4 was kindly provided by Upjohn Diagnostics (Kalamazoo, MI).

3. RESULTS

3.1. *LTB_4 stimulation of O_2^- production*

Addition of LTB_4 to alveolar macrophages resulted in a rapid, superoxide dismutase inhibitable, stimulation of O_2^- production (fig.1). Maximum initial rates of O_2^- production were 13.7 ± 2.6 nM $O_2^-/10^6$ cells per 5 min (mean ± SE, n = 4), which is similar to the maximal rates of O_2^- production achieved with formyl peptides [14]. The ED_{50} for LTB_4 stimulation of O_2^- production was determined to be approx. 3 nM. The time course of LTB_4-stimulated O_2^- production was

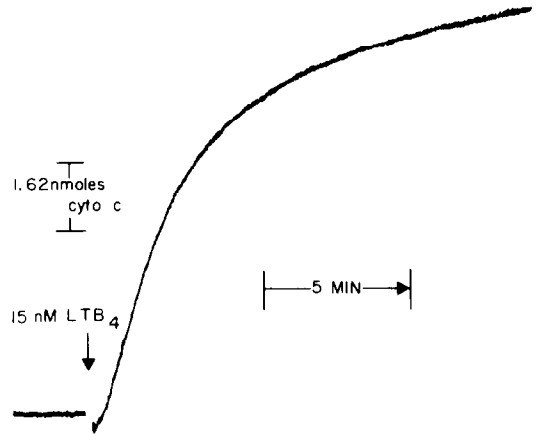

Fig.1. LTB_4-stimulated alveolar macrophage O_2^- production in MHBSS. Superoxide anion measurements (10^6 cells/ml) were conducted as described in section 2. LTB_4 (15 nM) was added as shown. The trace represents 5 sets of measurements.

observed to be slightly shorter in duration than for formyl peptide stimulation in MHBSS [14].

3.2. *Effect of LTB_4 on PI cycle activity*

The effect of 15 nM LTB_4 on PI cycle activity was examined in macrophages prelabelled with $^{32}P_i$ for 1 h at 21–23°C (fig.2). Addition of LTB_4 to $^{32}P_i$-prelabelled macrophages resulted in the rapid and significant loss of label in PIP and PIP_2 and a significant appearance of label in PA. The amount of label in PIP_2 returned to prestimulated values in 1 min, while the label in PIP required approx. 5 min to return to prestimulated values. The appearance of the ^{32}P label in PA was maximal within 1 min following stimulation which is consistent with the time course of changes in PIP_2. This pattern of changes in phospholipid labelling is similar to earlier reports on the effects of FNLLP [10] and hexachlorocyclohexane [15] on macrophage PI cycle activity and is in contrast to the lack of effect of LTB_4 on the PI cycle reported in PMN [1].

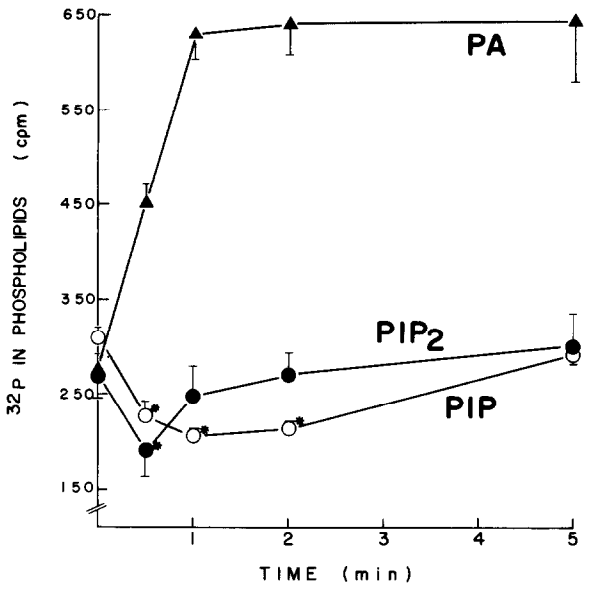

Fig.2. LTB_4-stimulated changes in the ^{32}P label in alveolar macrophage phospholipids at 21–23°C. Cells (1.5×10^6/ml) were loaded with 80 μCi/ml $^{32}P_i$ for 60 min and analyzed for labelled phospholipids after stimulation as described in section 2. LTB_4 (15 nM) was added at zero time. The points represent the mean (± SE) of 3 experiments. Asterisks indicate those values of PIP_2 and PIP that were significantly different ($P \leq 0.05$, Student's t-test) from their respective controls (zero time). All values of PA following LTB_4 addition were significantly elevated ($P \leq 0.05$, Student's t-test) over prestimulated values (zero time).

The ability of LTB_4 to stimulate the hydrolysis of PIP_2 was also verified by monitoring the appearance of ^3H-labelled 1,2-diacylglycerol (1,2-DAG) in macrophages preincubated with [^{3}H]arachidonic acid. Preliminary experiments indicated that under these labelling conditions [^{3}H]arachidonic acid was incorporated into PIP and PIP_2 in addition to other lipids. The changes in 1,2-DAG labelling upon the addition of 15 nM LTB_4 are shown in fig.3. Within 15 s following the addition of LTB_4 there is a significant increase in labelled 1,2-DAG which returned to prestimulated levels in 1 min. This time course of appearance of 1,2-DAG is consistent with the loss of PIP_2 and appearance of PA as described above.

LTB_4 also stimulated calcium mobilization in macrophages when measured either by using quin-2 loaded cells or as stimulated efflux of $^{45}Ca^{2+}$ from $^{45}Ca^{2+}$ preloaded cells (not shown). This is similar to the effects of LTB_4 on calcium mobilization in PMN [1] and formyl peptide stimulation of macrophages [14].

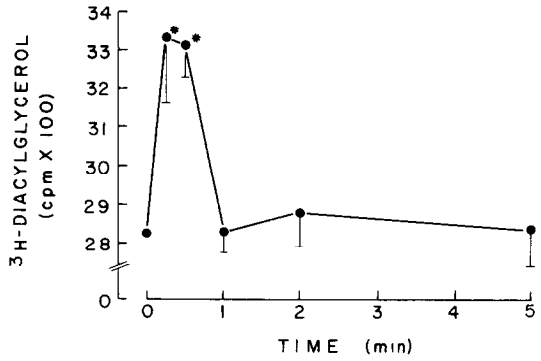

Fig.3. LTB_4-stimulated elevation of 1,2-DAG in alveolar macrophages at 21–23°C. Cells (4.5×10^6) were preincubated with 10 μCi [^{3}H]arachidonic acid for 1 h as described in section 2. LTB_4 (15 nM) was added at zero time. The points represent the mean (± SE) of 3 experiments (values at zero time were normalized to account for differences in extent of incorporation of [^{3}H]arachidonic acid). Asterisks indicate those values (15 and 30 s) that were significantly elevated ($P \leq 0.05$, Student's t-test) over values 1 min and after.

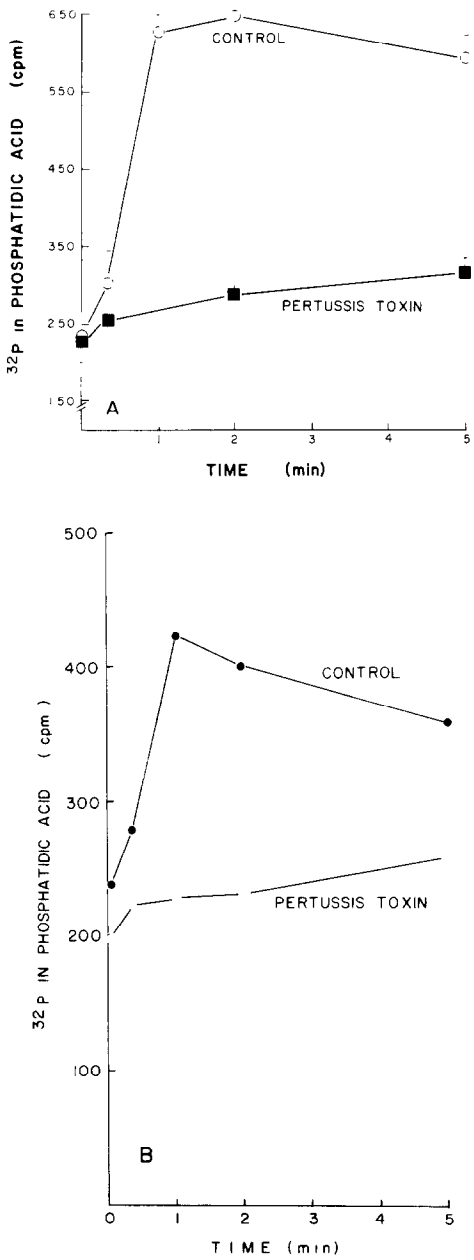

Fig.4. FNLLP- and LTB_4-stimulated changes in the ^{32}P label in PA for untreated cells and cells pretreated for 60 min at 37°C with 1 μg/ml pertussis toxin. Conditions were the same as described in fig.2. (A) FNLLP-stimulated changes for untreated cells (○) and pertussis toxin treated cells (■). The points represent the mean (± SE) of 3 experiments. (B) LTB_4-stimulated changes for untreated cells (●) and pertussis toxin treated cells (○). The points represent the average of data from 2 duplicate experiments. FNLLP (0.5 μM) and LTB_4 (15 nM) were added at zero time.

3.3. *Effect of pertussis toxin on PI cycle activity and O_2^- production*

Pretreatment of macrophages for 1 h with 1 μg/ml pertussis toxin at 37°C resulted in a 79.8 ± 5.6% (n = 4) inhibition of 0.5 μM FNLLP and >99% (n = 3) inhibition of 15 nM LTB_4 stimulated O_2^- production. The effects of pertussis toxin on FNLLP and LTB_4 stimulated PI cycle activity are shown in fig.4A and B, respectively. Parallel with the effects of pertussis toxin on O_2^- production, pertussis toxin treatment resulted in a significant inhibition of FNLLP or LTB_4 stimulated appearance of the ^{32}P label in PA.

4. DISCUSSION

LTB_4 stimulates PI turnover, Ca^{2+} mobilization and O_2^- production in a similar manner to that described for FNLLP stimulation of macrophages [10,14]. Stimulated PI turnover via activation of phospholipase C was verified by a similar time dependency (≤1 min) in the loss of PIP_2, formation of 1,2-DAG and conversion of 1,2-DAG to PA. These results also suggest that the conversion of 1,2-DAG via DAG kinase to PA is very rapid. In addition, PI turnover and O_2^- production stimulated by either LTB_4 or FNLLP could be blocked by pretreatment of cells with pertussis toxin which is proposed to act at a 41 kDa coupling protein between certain surface receptors and phospholipase C [8]. The similarities in results between exposure of macrophages to FNLLP and LTB_4 suggests that both stimulants transduce ligand-receptor binding through a common (pertussis toxin inhibitable) coupling protein to activate phospholipase C which hydrolyzes PIP_2 to yield diacylglycerol and inositol-1,4,5-trisphosphate. The production of diacylglycerol can result in the activation of protein kinase C and subsequent O_2^- production, while the production of inositol-1,4,5-trisphosphate has been reported to release Ca^{2+} from an intracellular store(s) [16].

The concentrations of LTB_4 required to elicit these changes in alveolar macrophages are similar to those required to stimulate O_2^- production in PMN [4]. Therefore, the reason for reported lack of stimulation of PI cycle in PMN [1] is not clear, but is remarkably distinct from those reported here for the macrophage. It could be predicted, based on the various reported observations in the PMN

of pertussis toxin inhibition and transient Ca^{2+} mobilization by LTB_4, that activation of PI cycle activity should also occur upon stimulation with LTB_4.

LTB_4 is a lipoxygenase product that has been shown to be produced in large quantities by the alveolar macrophage [2]. The released LTB_4 can serve to recruit additional inflammatory cells to the site of inflammation in the lung and to directly activate O_2^- production by the alveolar macrophage. These results demonstrate that LTB_4 is a potent stimulant of alveolar macrophage O_2^- production, most probably via a mechanism very similar to that proposed for the chemotactic formyl peptides. Furthermore, these results provide another example of the probable association between a coupling protein and PI turnover leading to Ca^{2+} mobilization and O_2^- production during macrophage activation.

ACKNOWLEDGEMENTS

This work was supported by the following grants from the National Heart, Lung and Blood Institute: HL-34227 and HL-33953.

REFERENCES

[1] Volpi, M., Yassin, R., Tao, W., Molski, T.F.P., Naccache, P.H. and Sha'afi, R.I. (1984) Proc. Natl. Acad. Sci. USA 81, 5966–5969.
[2] Bonney, R.J., Opas, E.E. and Humes, J.C. (1985) Fed. Proc. 44, 2933–2936.
[3] Lewis, R.A. and Austin, K.F. (1984) J. Clin. Invest. 73, 889–897.
[4] Serhan, C.N., Radin, A., Smolen, J.E., Korchak, H., Samuelsson, B. and Weissmann, G. (1982) FEBS Lett. 107, 1006–1012.
[5] Goldman, D.W. and Goetzl, E.J. (1984) J. Exp. Med. 159, 1027–1041.
[6] Serhan, C.N., Fridovich, I., Goetzl, E.J., Dunham, P.B. and Weissmann, G. (1982) J. Biol. Chem. 257, 4746–4752.
[7] Goldman, D.W., Chang, F.-H., Gifford, L.A., Goetzl, E.J. and Bourne, H.R. (1985) J. Exp. Med. 162, 145–156.
[8] Okajima, F., Katada, T. and Ui, M. (1985) J. Biol. Chem. 260, 6761–6768.
[9] Dauber, J.H. and Daniele, R.P. (1978) Am. Rev. Resp. Dis. 117, 673–684.
[10] Holian, A. and Stickle, D.F. (1985) J. Cell. Physiol. 123, 39–45.
[11] Holian, A. and Daniele, R.P. (1979) FEBS Lett. 108, 47–50.
[12] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[13] Imai, A., Nakashima, S. and Nozawa, Y. (1983) Biochem. Biophys. Res. Commun. 110, 108–115.
[14] Stickle, D.F., Daniele, R.P. and Holian, A. (1984) J. Cell. Physiol. 121, 458–466.
[15] Holian, A., Marchiarullo, M.A. and Stickle, D.F. (1984) FEBS Lett. 176, 151–154.
[16] Streb, H., Irvine, R.E., Berridge, M.J. and Schulz, I. (1983) Nature 306, 67–69.